

THEREFORE WHAT IS CLAIMED IS:

1. A sensing element for use in a light diffraction assay for detecting the presence or absence of at least two analytes, comprising:
a substrate including a surface and having on said surface a first pattern comprising a first analyte-specific receptor and at least a second pattern comprising a second analyte-specific receptor, each said pattern corresponding to a diffraction pattern distinct from each other.
2. The sensing element according to claim 1 wherein said at least two patterns interpenetrate each other in a preselected area on said substrate surface.
3. The sensing element according to claim 1 wherein said at least two patterns are located in close proximity to each other but do not interpenetrate each other on said substrate surface.
4. The sensing element according to claim 1 wherein said at least two patterns are formed within a selected area on said surface, including a plurality of selected areas on said surface of said substrate with each selected area of said substrate surface having thereon at least two patterns.
5. The sensing element according to claim 1 wherein said analyte-specific receptors are one of a member of a binding pair selected from the group consisting of antibody-antigen, enzyme-inhibitor, complementary strands of nucleic acids or oligonucleotides, receptor-hormone, receptor-effector, enzyme-substrate, enzyme-cofactor, glycoprotein-carbohydrate, binding protein-substrate, antibody-hapten, protein-ligand, protein-nucleic acid, protein-small molecule, protein-ion, cell-antibody to cell, small molecule-antibody to said small molecule, chelators to metal ions and air-borne pathogens to associated air-borne pathogen receptors.

6. The sensing element according to claim 1 wherein one of the analyte-specific receptors assays for a known standard that is present in a medium to be screened for said analytes.
7. The sensing element according to claim 1 wherein said analyte-specific receptors include surface relief patterns formed directly in the surface of said substrate.
8. The sensing element according to claim 1 including an intervening layer located directly on said surface of said substrate, and wherein said analyte-specific receptor patterns are located on said intervening layer.
9. The sensing element according to claim 8 wherein said intervening layer is a layer of avidin in a pattern, and wherein said analyte-specific receptors are biotinylated analyte-specific receptors to bind with the patterned avidin layer.
10. The sensing element according to claim 1 wherein said substrate is selected from the group consisting of glass, silanized glass, silicon, silicon dioxide, polymer, metal, metal oxide, metal film, metal oxide film, partially or fully reflective substrates including metals, and metal coated substrates.
11. The sensing element according to claim 1 wherein said substrate includes opposed surfaces, including analyte-specific receptor patterns formed on both of said opposed surfaces.
12. The sensing element according to claim 1 wherein said substrate is a dipstick.
13. The sensing element according to claim 1 wherein said substrate has encoded thereon instructions that identify which analyte-specific receptors are

present in said at least two patterns.

14. A diffraction binding assay method for detecting simultaneously at least two analytes, in a medium, comprising:

providing a substrate including a surface and on said surface a first pattern of a first analyte-specific receptor and at least a second pattern comprising a second analyte-specific receptor, each said pattern corresponding to a diffraction pattern distinct from each other;

contacting said surface of the substrate with the medium for a sufficient time to permit preselected analytes present in solution to bind with their analyte-specific receptors; and

illuminating said substrate and detecting light diffracted from said substrate surface and analysing said diffracted light for presence of a diffraction image representative of binding of one or more analytes with their analyte-specific receptors and identifying from said diffraction image one or more analytes present in said medium.

15. The method according to claim 14 wherein illuminating said substrate includes illuminating a sufficient area of said substrate to illuminate at least a part of each of said at least two patterns.

16. The method according to claim 14 wherein illuminating said substrate includes illuminating the patterns one a time.

17. The method according to claim 15 wherein analysing said diffracted light for the presence of a diffraction image representative of binding of one or more analytes with their analyte-specific receptors includes storing the diffraction image from said illuminated area.

18. The method according to claim 15 including detecting light diffracted from said substrate surface prior to exposure of said substrate surface to said medium

for producing a baseline diffraction image due to said substrate and analyte-specific receptor patterns in the absence of analytes, including storing said baseline diffraction image.

19. The method according to claim 18 wherein analysing said diffracted light for the presence of a diffraction image representative of binding of one or more analytes with their analyte-specific receptors includes comparing said diffraction image with said baseline diffraction image.

20. The method according to claim 15 wherein said light source is a laser that emits a substantially coherent, monochromatic laser beam.

21. The method according to claim 20 wherein said laser emits light in the infrared, visible or ultraviolet.

22. The method according to claim 14 wherein said substrate is substantially transparent and said surface is illuminated from one side of said substrate, and wherein said light diffracted from said substrate is detected on the opposite side of said substrate.

23. The method according to claim 14 wherein said substrate is partially reflecting and said surface is illuminated from one side thereof, and wherein diffracted light is detected on the same side of said substrate.

24. The method according to claim 14 wherein said substrate is reflecting, and said surface is illuminated from one side thereof, and wherein diffracted light is detected on the same side of said substrate.

25. The method according to claim 14 wherein after contacting said surface of the substrate with a medium being screened for preselected analytes said substrate is rinsed and dried prior to being illuminated.

26. The method according to claim 14 wherein contacting said surface of the substrate with the medium includes placing said substrate in a cell containing said medium being screened for analytes, said cell having at least one optical window for light to pass therethrough for detecting for analytes in said medium *in situ*.

27. The method according to claim 26 wherein intensities of selected regions of the resulting diffraction image are monitored as a function of time.

28. The method according to claim 14 wherein the light illuminating said substrate is directed toward said substrate at an effective angle such that it undergoes total internal reflection from the substrate/medium interface.

29. The method according to claim 14 wherein said analyte-specific receptors are one of a member of a binding pair selected from the group consisting of antibody-antigen, enzyme-inhibitor, complementary strands of nucleic acids or oligonucleotides, receptor-hormone, receptor-effector, enzyme-substrate, enzyme-cofactor, glycoprotein-carbohydrate, binding protein-substrate, antibody-hapten, protein-ligand, protein-nucleic acid, protein-small molecule, protein-ion, cell-antibody to cell, small molecule-antibody to said small molecule, chelators to metal ions and air-born pathogens to associated air-born pathogen receptors.

30. The method according to claim 14 wherein said substrate is selected from the group consisting of glass, mica, polished silicon, silicon dioxide, polymeric materials, substantially transparent polymeric materials, partially or fully reflective substrates including metals, and metal coated substrates.

31. The method according to claim 14 including contacting said surface of the substrate with a medium containing a standard material that binds to the bound analytes after contacting said surface of the substrate with the medium being screened and prior to illuminating said selected area of said surface.

32. The method according to claim 31 wherein said standard material is selected from the group consisting of proteins, metal colloids, polymer colloids, colloidal silica, quantum dots, or combinations thereof.

33. The method according to claim 14 wherein the medium is selected from the group consisting of blood, serum, plasma, urine.

34. An apparatus for detection of analytes in a medium using diffraction of light, comprising:

a source of illumination;

a sensing element including a substrate having a surface and on said surface a first pattern of a first analyte-specific receptor and at least a second pattern comprising a second analyte-specific receptor, each said pattern corresponding to a diffraction pattern distinct from each other, said source of illumination being positioned so as to illuminate said substrate surface;

detection means positioned with respect to said sensing element to detect light diffracted from said illuminated surface; and

processing means for analysing said diffracted light for presence of a diffraction image representative of binding of one or more analytes with their analyte-specific receptors and identifying from said diffraction image one or more analytes present in said medium.

35. The apparatus according to claim 34 wherein said processing means is connected to said detection means and includes storage means for storing signals that are output from said detection means corresponding to said diffracted light, said processing means including image analysis means for deconvoluting said diffraction image.

36. The apparatus according to claim 35 wherein said processing means includes signal processing means for calculating kinetics of interaction of said analytes binding with their analyte-specific receptors from said diffracted light

stored as a function of time.

37. The apparatus according to claim 34 wherein the source of illumination produces a coherent and monochromatic collimated beam of light.

38. The apparatus according to claim 34 wherein said source of illumination is a laser with emission at UV, visible, near-infrared or infrared wavelengths.

39. The apparatus according to claim 34 wherein said light illuminating said substrate surface is delivered through an optical fiber.

40. The apparatus according to claim 34 including a cell enclosing a chamber to contain therein said medium being screened for analytes, said cell being adapted to receive said substrate with said selected area of said substrate in contact with said medium, said cell having at least one optical window for light to pass therethrough for detecting for analytes in said medium *in situ*.

41. The apparatus according to claim 40 wherein the light used to illuminate said surface of said substrate undergoes total internal reflection from the substrate/medium interface.

42. The apparatus according to claim 40 wherein said cell includes a fluid inlet and a fluid outlet for continuous flow of said medium through said cell.

43. The apparatus according to claim 34 wherein the substrate is selected from the group consisting of glass, silanized glass, silicon, silicon dioxide, polymer, metal, metal oxide, metal film, metal oxide film, partially or fully reflective substrates including metals, and metal coated substrates.

44. The apparatus according to claim 34 wherein said detection means is an imaging device.

45. The apparatus according to claim 44 wherein said imaging device is an electronic imaging device.

46. The apparatus according to claim 44 wherein said imaging device is a camera.

47. The apparatus according to claim 34 wherein said detection means includes one of a photodiode detector, a photomultiplier tube, an avalanche photodiode and a position-sensitive photodiode.

48. The apparatus according to claim 45 wherein said imaging device is a matrix array detector.

49. The apparatus according to claim 48 wherein said matrix array detector is a CCD detector array.

50. The apparatus according to claim 34 wherein said analyte specific receptors are one of a member of a binding pair selected from the group consisting of antibody-antigen, enzyme-inhibitor, complementary strands of nucleic acids or oligonucleotides, receptor-hormone, receptor-effector, enzyme-substrate, enzyme-cofactor, glycoprotein-carbohydrate, binding protein-substrate, antibody-hapten, protein-ligand, protein-nucleic acid, protein-small molecule, protein-ion, cell-antibody to cell, and small molecule-antibody to said small molecule, chelators to metal ions and air-born pathogens to associated air-born pathogen receptors.

51. The apparatus according to claim 34 wherein one of the analyte-specific receptors assays for a known analyte that is present in a medium to be screened for said analytes.

52. The apparatus according to claims 34 wherein said analyte-specific

receptors are laid out in said patterns directly on the substrate.

53. The apparatus according to claim 34 including an intervening layer formed directly on the surface of said substrate, and wherein said analyte-specific receptor patterns are laid out on said intervening layer.

54. The apparatus according to claim 53 wherein said intervening layer is a layer of avidin in a pattern, and wherein said analyte-specific receptors are biotinylated analyte-specific receptors to bind with the patterned avidin layer.

55. A method of producing a sensing element for use in a light diffraction assay, comprising:

depositing within a selected area on a surface of a substrate a first pattern of a first analyte-specific receptor immobilized thereon and at least a second pattern comprising a second analyte-specific receptor immobilized thereon, each said pattern corresponding to a diffraction pattern distinct from the others.

56. The method according to claim 55 wherein said at least two patterns interpenetrate each other in a preselected area on said substrate surface.

57. The method according to claim 55 wherein said at least two patterns are deposited in closed proximity to each other but do not interpenetrate each other on said substrate surface.

58. The method according to claim 55 wherein each pattern is produced by mixing a material comprising the analyte-specific receptor in a liquid and printing each pattern onto said surface of said substrate followed by drying.

59. The method according to claim 55 wherein each pattern is produced by mixing a material comprising the analyte-specific receptor in a liquid and printing each pattern onto said surface of said substrate followed by exposure of each

pattern to an agent that covalently binds the material to the substrate.

60. The method according to claim 59 wherein the agent is light.

61. The method according to claim 53 wherein the printing of the patterns is accomplished by microcontact printing using an elastomeric stamp having surface topography corresponding to said patterns.

62. The method according to claim 55 wherein said patterns are laid down using inkjet printing, and using robotic manipulation.

63. The method according to claim 55 including depositing an intervening layer directly on said surface of said substrate, and wherein said analyte-specific receptor patterns are deposited on a top of said intervening layer.

64. The method according to claim 63 wherein said intervening layer is a layer of avidin in a pattern, and wherein said analyte-specific receptors are biotinylated analyte-specific receptors to bind with the patterned avidin layer.

65. The apparatus according to claim 34 including means for rastering said light source across the surface of said substrate.

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